APPLICATION OF AMNIOTIC MEMBRANE IN NEGLECTED CASES OF BURN

THESIS
FOR MASTER OF SURGERY
(GENERAL SURGERY)

BUNDELKHAND UNIVERSITY
JHANSI
1985

PREM MOHAN

DEPARTMENT OF SURGERY
M.L.B. MEDICAL COLLEGE
JHANSI.

C E R T I F I C A T E

Certified that the work entitled " APPLICATION OF AMNIOTIC MEMBRANE IN NEGLECTED CASES OF BURN " has been carried out by DR. PREM MOHAN himself in this department.

He has put in the necessary stay in the department as required by the regulations of Bundelkhand University.

(S. L. AGARWAL)
M.S., F.R.C.S.
Professor and Head,
Department of Surgery,
M.L.B.Medical College,Jhansi.

Dated: 28.7.84

DEPARTMENT OF SURGERY
M.L.B. MEDICAL COLLEGE
JHANSI.

C E R T I F I C A T E

Certified that the work entitled " APPLICATION OF AMNIOTIC MEMBRANE IN NEGLECTED CASES OF BURN " has been carried out by DR. PREM MOHAN under my constant supervision and guidance. The results and observations were checked and verified by me from time to time.

This thesis fulfils the basic ordinances governing the submission of thesis for M.S., laid down by Bundelkhand University.

(R. P. KALA)
M.S.
Reader,
Department of Surgery,
M.L.B. Medical College, Jhansi.

Dated: 28-7-84

DEPARTMENT OF OBSTETRICS & GYNAECOLOGY
M.L.B. MEDICAL COLLEGE
JHANSI.

C E R T I F I C A T E

This is to certify that DR. PREM MOHAN has worked on " APPLICATION OF AMNIOTIC MEMBRANE IN NEGLECTED CASES OF BURN " under my guidance and supervision. His results and observations have been checked and verified by me from time to time.

(R. MITRA)
M.S., D.G.O.
Professor and Head,
Department of Obstetrics & Gynaecology
M.L.B. Medical College, Jhansi.

Dated: 28-7-84

A C K N O W L E D G E M E N T

I shall remain indebted to Prof. S.L. Agarwal,M.S., F.R.C.S. for his close supervision, concrete suggestion and constructive criticism. I feel highly obliged to Dr.R.P.Kala, M.S. Reader in the department of Surgery, for his worthy guidance unlimited help and persistant encouragement went a long way in completion of this work.

I shall be highly grateful to Dr.Rama Mitra, M.S.,D.G.O. for her time to time assistance and valuable advice.

I am really thankful to my colleagues in the department of surgery and department of Obstetrics & Gynaecology for their kind assistance.

My special thanks to Dr.N.K.Gupta and others in the department of Anaesthesia, for providing general analgesia to my patients off & on, even in odd hours and prevented the patients from intolerable pain during debridement and cleaning.

Mr. B.P.Tiwari deserves a special note of thanks for painstaking job of converting the manu-script to tran-script.

In the last, but not the least I must mention the constant moral support of my family members.

Prem Mohan

(PREM MOHAN)

CONTENTS

***
*

INTRODUCTION

INTRODUCTION

Since man first learned to make fires, burns have caused great suffering to the mankind medically, socially as well as economically. With the advancement of science, in the field of machine and chemical industry the use of electrical energy and radiation in day to day life ,the susceptibility for burn has increased tremendously. In larger cities of India, such as Bombay most children and young people die of burns than from acute infectious diseases. About 74% are domestic burns and 79% of all domestic burns involve women and children.

The treatment of burn is complex, expensive and requires great collective team effort. During the past decade or so the local treatment of burn was either the " "Open " or the " Closed " method which because of the unfavourable conditions in the hereby environment almost invariably led to infection. We can realize the ordeal that a burn patient suffers during his daily dressing not to mention the repulsive smell that he has to constantly conted with and what of the time, effort and money spent in the care of these patients. Statistics show that the overall mortality rate of burns is the same now as it was 20 years ago, in spite of the advant of antibiotics and the advancement in the knowledge of fluids and electrolyte

therapy. The present work is an endevour with the use of foetal membrane homografts perse to improve morbidity figures or at least make the individual patient comfortable before be finally leaves the hospital homeward or heaven ward.

The basic problems of a burn patient are (1) pain (2) infection (3) autointoxication and the loss of fluid, proteins and electrolytes, the last being the most serious and immediate problem. All these except auto intoxication are caused by the destruction of the highly protective epidermal covering of the body in second degree and deeper burns.

Various materials have been used by various workers at different times either biological or synthetic. Different biological coverings are homograft skin, heterografts skin, collagen sheet, foetal membrane (Amnion and/or chorion). Synthetic materials include solid silicans polymer membrane, micro porous material, cotton gauge, sponge, febric spong, gels & laminates. Accepting the most ideal way to use autografts skin but it is not always possible if burn area is extensive with lack of donor site and also patient is not fit for extensive surgery.

Pain is caused by the irritation of exposed nerve ending by the clothing dressing or even the mere contact of air. This can not be completely controlled by medications but can be reduced to great extent by avoiding daily dressing after application of some biological or synthetic covering.

Infection is a great problem in neglected cases of burn which has already occupied the resistant strains of bacteria. This can be controlled by the use of antibiotic after culture and sensitivity of the pus and also by local cleaning of raw area. Once the infection is controlled further infection can be prevented by some barrier covering. This saves the unnecessary expenses of patient on costly medicines for long duration and prevents the regards of antibiotics. This infection also interferes with and delays the normal healing process resulting into deformities, contractures cicatrices and scars.

The problem of loss of fluids, proteins and electrolyte is caused by the rapid oozzing from the raw body surface. It can be treated by the use of necessary fluids, proteins and electrolyte replacement. In addition their loss can be greatly minimized by covering the raw surface.

In ancient days, the treatment of burn wounds was only local application of various medicinal products but with the knowledge of metabolic changes in body secondary to discharge and infection of raw burn area has lead to the choice of treatment by closed method.

Amniotic membrane has been used in fresh cases of burns with the idea of avoiding pain, electrolyte imbalance, infection etc. In the present work the aim of application is different in the sense that material cost of the treatment is minimum, early granulation & healing & to

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Even with the recent advances in medical sciences problem of burn management is still a threat to the life. These injuries either due tc fire, radiation, electricity or chemical agents have grown more important socially, medically, and economically with every coming year due to change in the life style of mankind.

As far back as 1500 B.C.Papyrus had used cow-dung topically. Ancient Indian literature shows that Sushtra used mixture of butter with red achre or the bark of a fig tree. He also debrided severe burns with loose skin and flesh. Around 5th - 6th centuries, B.C. the Egyptians were treating burns by inceneration and a mixture of gum, goat's hair and milk from a lady who has given birth to a son. Chinese and Japanese were using tinctures and extracts made from tea leaves around 430 B.C.

Adams (1939) reports that Hippocrates applied warm mixture over the burn and avoided suppuration by simple cleanliness. Paulus aeginata (AD 625-690) recommended application of moderate detergent material which were not definitely heating or cooling.

In Ancient Rome three methods were used

1. Celsus suggested mixture of honey and bran.
2. Pliny and Elder suggested exposure method.

3. Galen suggested local application of venegar or wine over burn surface.

In 7th century A.D. Paulus of Aegina used the various emolient preparations. Rhazes (AD 580-923) had been using white ointment composed of white lead,oil of roses and wax. He also used ice cold water locally. Apart from excision of contracted scars described by celsus, surgery had no place in the treatment of burns with Greek and Romans. Volesco de Tarenta of Montpellier (1490) described method to avoid syndactyly in the burnt hands.

Ambrose Pare (1517-1590) suggested ointments for the treatment of burn wounds. Claws (1591) used 5 different complex preparations on the different parts of body area involved in burn. He stands out in history as the first Surgeon since the middle ages to use the physical signs of burns ' where the skin was burnt off, and the parts w-ere made raw and painful ' to indicate his local treatment.

Gailhelmus Febricus Hildanus (1607), the father of German surgery, described 3 degrees of burn according to depth. L. Heister (1683-1758) classified the burns into four degrees according to depth and including time factor.

David cleghron (1792) used the Venegar and Chalk poultices locally. Edward Kentish (1797) suggested the pressure dressings to relieve pain and to stop blisters formation.

Sir James Earle (1799) suggested the ice cold water and told that it acts as a good analgesic and prevents oedema formation. Syme (1827) suggested the use of dry cotton wool dressing with firm pressure.

Boyer (1814) classified burns into three degrees: Erythema, Blistering leading to superficial ulcers and eschar. Dupaytren (1932) classified the burn into 6 degrees according to depth of involved tissue.

1. Erythema or superficial philogosis which blenches in pressure.
2. Cutaneous inflammation, with the loss of epidermis and the development of vesicles filled with serum.
3. The destruction of a portion of the papillary body.
4. The disorganization of the whole dermis to a subcutaneous cellular tissue.
5. The formation of eschars of all the carbonization of the whole thickness of the burnt part.

He also described the 4 periods during the natural course of burn injury.

1. Period of irritation 2. Period of inflammation
3. Period of suppuration 4. Period of Exhaustion

He also described the gastro intestinal haemorrhage in the burn cases. Later Curling (1842) recognized gastric and duodenal ulcers as a cause of gastro intestinal haemorrhage in burn cases.

Passowant (1858) suggested the use of saline bath, Copeland (1877) suggested the exposure method. Edward Clark Davidson (1894-1933) used tannic acid on burn surface in 1925. He suggested that these agents decreases the fluid loss, relieve pain and produce a clean scar. Later on Meclure in 1944 found this agent to be hepatotoxic and as a cause of many death in burn patients. Therefore, its use abondoned since that time. Addridge (1933) suggested the use of gention violet as escharotic agent on burn wound for cleaner scar. 5% silver nitrate was also used.

In 1942 Allen and Koch of chicago suggested the use of petroleum gauze piece to apply over burn wound with strict immobilization and abondoned the use of pressure bandages.

Wallece of Edinburg (1949) reintroduced the exposure method for treatment of burn in England. Pulaki, Artz and Blocker also started this method in united state of America in 1950. Later on other surgeons also accepted the same remedy with view that development of crust and scar provides physiological covering to burn wound and in this way reduce the disadvantages of raw areas in burns.

Leidbug, Reiss and Artz (1953) pointed out that septicaemia was the primary cause of death in burns and many of the deaths were due to staphylococci. As improved antibiotics against the gram positive organism become

available, pseudomonas sepsis became very common and was considered as primary cause of death. To reduce the growth of bacteria under the burn surface different agents like 5% $AgNo_3$ (Moyer) Mefemide or sulfamylon (Moncrief), Silver sulfadiazine (Fox C.L.Jr. Rappole B.W. Stanford W, (1969) cerium nitrate (William W. monofo, Som N. Tandon), Cerium nitrate and silver sulfadiazine (Fox C.L.Jr. 1975) were suggested. But these topical agents are effective marely in control of bacterial population of burn wound, not in sterilization.

Burn Wound Coverings

Effects of thermal injuries are due to immediate traumatic shock and loss of skin covering leading to anatomic, metabolic and physiological disturbances. Traumatic shock is not a significant contributing factor to mortality and morbidity due to burns now-a-days, because up to 80% of burnt area shock can be managed successfully. The main cause of morbidity and mortality is toxaemia due to absorption of poisons from the injured surface or from loss of skin covering leading to abnormal loss of heat and body constituents, and invasion of micro-organism. So the new concept of burn care is restoration of impaired barrier. Autogenous skin grafting is the best coverage material amongst all, suggested till this time, but it has its own limitations, in form of limited supply. Unfitness of the already shocked

patient for surgical procedure involved in skin grafting and the refusal of patients or his attendent on religious, sentimental or ethical ground. To overcome this problem, various biological and synthetic coverage materials wether for short period till the healing of wound or permanent in place of lost skin, has been suggested by different workers.

Biological Dressings

Homografts- About a century ago, Pollack (1871) applied the first homograft on a burn patients. In 1881 Girdner treated a lightening burn with the skin from a suicide victim. Shede used skin from amputation specimen as well as from cadavers with the limit of 24 hours. Ivunova in 1890 stressed the use of foetal skin as a homograft on burn surface because of its more energetic vitality.

In 1952 Dogo of Italy used the post mortam allografts as a temporary biological dressings. He noted that cadaver skin is useful when obtained especially while it is still viable and where it is possible to preserve the tissue till the time of application. He prooved skin viability by determining the tissue oxygen uptake in the worburg apparatus. The skin was preserved at 3°c in physiological solution. The oxygen consumption of cadaver skin was noted unaffected upto 16 hours after death.

Brown (1952) reported the use of allografts as emergency dressing for burns. He stated that skin may be removed even days after death if cadaver has been placed in cold storage.

Eade (1958) and Morries (1960) observed that the homografts have organizational and debridemental effects on healing wound. They pointed out that the count of bacteria on granulating infected wound decreases within 2 hours of homografts application.

In 1967, Miller et al studied the use of frequently changed skin homografts to promote healing in non healing infected ulcers. He observed that in 2nd degree burns if healing occurs without homografts, epidermis shows the alterations in the architecture and is disorganised and dermis contains oedematous connective tissue, while if healing occurs under the coverage of homografts, the epidermis shows normal architecture with recognizable basal layer and normal collagen bundles in the dermis.

Sharma et al (1978) used the preserved homografts in 25 cases out of which 15 were of the superficial burns. The period of normal survival of grafts over wound surface was 11 days if recipient and donar's blood groups was same. Where blood groups was not same, it was 13 days.

Allograft skin, even being satisfactory biological dressing, have their limitations. It has limited supply and personnel are required for procurement. Boxter (1970) has estimated that six physician hours and hospital cost of $ 225 per patient are needed to use cadaver allografts.

Xenografts:

Because of limitations in the use of homografts, xenografts came in use. Brown, Burleson and Tavis have shown that the adherence of allografts and xenografts is similar. Hetrografts provides a readily available easily stored and sterilized dressing in contrast to homografts. In 1960, canine skin has been used by Switzer et al. Porcine skin is the xenograft material of choice however, and Bromberg et al and Elliott and Hoehn have used pig skin. Variable results have been reported from early re-epitheliazation to conversion of full thickness skin loss. Salisburg (1973) has reported some poor results, when they used this type of dressing on donor sites, with increased inflammation and delayed repair following treatment, Comparative experiments have shown no significant difference in the effectiveness of fresh compared with fresh frozen or forzen irradiated porcine skin.

The most striking advantage with the porcine xenograft is that of immediate and lasting pain relief. Xenograft has most of the properties of the ideal skin substitute. A viable xenograft is antigenic but the dead is not. The major problem is the propensity to digestion by wound collagenase and subsequent infection.

Collagen Sheet

Collagen is a fibrous protein which is present in many animal tissues like skin, muscle and bone. When implanted in

living animals, tissue in pure form, it does not produce any antigenic reaction. Collagen sheets are derived from serous and subserous layers of freshly slaughtered cattle intestine. These are available in 4" x 6" size and packed in cylindrical glass tubes containing ethylene oxide which acts as sterilizing agent.

Sinha (1972), Shanker (1975) and Gupta et al (1976) used collagen sheets as primary cover material in management of burns. Gupta and Chaturvedi (1974) used it to cover donar areas. Thukral and Gupta (1976) have used collagen material in repair of hernia and to cover surgical defects. Ilhans et al (1978) used sheets as biological dressing in 32 patients and reported its role in prevention of infection and in increasing the rate of healing. Jain et al (1976) reported the similar findings.

The effects of collagen sheet are, a) prevention of air born infection, b) minimising fluid loss, c) promoting formation of healthy and pink granulation tissue. But it is expensive material and is not available at every centre.

Synthetic Materials

Pickrell (1942) worked on sulfonamide film. Many of these materials adhere by intrapment of coagulum, in the interestices of the material. Silicon polymer membrane is the best material available because it is elastic, durable and the water vapour transfer characteristics can be controlled by varying the thickness. Kornberg et al (1977) have used thin

silicon membrane bondedto cotton gauze for temporary skin substitution but it lacks elasticity and creats non uniform pattern of adherence. Other materials are modifed polyvanyl chloride or similar plastics which provides more elasticity and water vapour transfer characteristics. (James et al 1975; Lamkey et al 1977; Towsend, 1977). The material is deep but the greatest disadvantage is lack of adherence to wound itself. These materials seem to have great promise as a temporary skin substitute for short time applications.

Amniotic membrane

It is most economic and freely available biological dressing, have most of properties of ideal skin substitute.

Amnion is the inner one of two foetal membranes. Its inner surface is in contact with contents of amniotic sac ie. amniotic fluid and foetal body. Its outer surface is separated from decidua of maternal uterus by chorion.

It has following anatomical parts :-

a- Placental amnion: Lines inner aspect of placenta

b- Reflected amnion: Lines rest of amnion

c- Dependent amnion: Overlies the internal os of cervix.

The amnion which is normally 0.02 to 0.5 mm in thickness, consists of five layers. These are from with-in outwards (A) Epithelium (B) Basement Membrane (C) Compact layer (D) Fibroblast layer and (E) Spongy layer.

(A) The Epithelium : This is the inner most layer in contact with the amniotic fluid. It consists of a single layer of cells which are usually cuboidal but may be columnar over the placenta or flattened to pavement cells on the reflected amnion. They normally contain a single nucleus and are densely adherent to the underlying basement membrane on their free, normally convex surface. They are surmounted by microvilli to form a brush border. The cells contain a number of vacuoles of varying size.

(B) The Basement Membrane : This is a thin layer composed of a network of reticular fibres and is well marked over both the placental and the reflected parts of the amnion. Short blunt processes from the bases of the epithelial cells inter digitate with similar process that arise from the basement membrane.

(C) The Compact Layer : This consists of a complex network of reticular fibres but devoid of cells. This layer which is probably the strongest of the amniotic layers is rarely thickned by oedema and it appears to resist, to some extent penetration by leukocytes.

(D) The Fibroblast Layer : This is the thickest layer of the amnion. It is composed of a loose fibroblast network embedded in a mass of reticulin. The cells occasionally show phagocytic activity.

(E) The Spongy Layer : The tissue of the extra embryonic celom is compressed between the amnion and the chorion to form the

spongy layer. These are wavy bundles of reticulin with branching fibres having triangular shaped nodes at the junctions. A few isolated fibroblasts are present in this layer. This layer frquently becomes oedematous and as such, accounts for the increase in thickness. It permite the amnion to slide upon the underlying chorion.

The amnion has no blood supply or lymphatic system. The nerve supply is still not confirmed.

Embryologic Development :

The development of amnion begins during the transformation of morula to blastocyst stage at the time of implantation, about 7-8 days after fertilization. There is separation from inner cell mass of the germ disk at the periphery of the ectodermal layer of polyhedral cells,"amniogenic cells " to form a slit like cavity with appearance of primary extra embryonic mesoderm. The amniotic epithelium becomes separated from the primitive trophoblast. Amniotic mesenchyme is derived from the primary extra embryonic mesoderm of the blastcyst.

Immunology :

Concerning this aspect following studies has been carried out when amnion was implanted to its own new born infants, It has been demonstrated that 'take' was as permanent graft. Amnion, not formed attachement to host tissue and mesenchyme cells, was kept towards host in those studies. Neovascularization was not occured.

Nourishment of graft appeared to be by simple diffusion.

When subcutaneous implantation was done of allograft amnion, results were same as autograft for first 14-17 days. Latter on these grafts were transformed into hyalinized substances. Only mild infiltration of round cells was observed by 20-30 days. When the amniotic membrane was used as biological dressing for surface defects as allografts and autografts same result has been observed. Superior 'take' or' fixation ' was noted when mesenchymal surface was placed towards host. When amnion was placed towards the host, little fixation was noticed at the end of 72 hours. No neovascularization was observed in any case.

When the allograft amniotic membrane was placed in pelvic cavity after pelvic extenteration, it was recovered at the end of 21 days and appeared viable histologically. Granulation tissue and fibroblastic tissue activities were markedly inhibited as compared to control cases.

When allograft amnion implantation was done, in intra peritoneal cavity, in the experimental animals, in whom the caecum was damaged and contaminated, prevention of adhesions add gradual disintegration of membrane without any host response were observed.

These experiments suggest that antigenicity of amnion is low and no voilent host reaction noted yet.

The Chorion :

The chorion consists of four layers. These are from within outward : (F) Cellular layer (G) reticular layer (H) pseudo basement membrane and (I) trophoplast.

(F) Cellular layer : This is a thin layer consisting of an interlacing fibroblast network. It is frequently imperfect or completely absent from the chorion when examined at term.

(G) Reticular layer : This forms the majority of the thickness of the chorion and consists of a reticular network, The fibres of which tend to be parallel. Nodes are present on the fibres at those places where branching occurs. A few fibroblasts are present together with many macrophages.

(H) Pseudo basement membrane :

This forms a type of basement membrane for the trophoblast. It is a layer of dense connective tissue that is firmly adherent to the reticular layer above and which sends anchoring and branching fibres down into the trophoblast.

(I) Trophoblast : It consists of from two to ten layers of trophoblast cells in contact, on their deeper aspect, with maternal decidua. This layer contains the obliterated chorionic villi.

The chorion contain branches of umblical vessel in the substances of its reticular layer but does not receive any capillary blood supply from them.

When the chorion was placed over host tissue as autograft, neovascularization and migration of host cells was observed, causing host versus graft rejection phenomenon. It provokes strong cellular and less antibody response. The tissue had an accelerated rejection phenomina in 72 hours and being rejected by 11th day. This rejection phenomina can be delayed by high dose progesterone.

Antigens have been demonstrated by a number of workers on trophoblastic cells. Recent studies shows that chorionic tissue may have 3 usual antigens, two from mature placenta and one relatively specific for chorion.

Clinical and Experimental application

John Staigu Davis, at John Hopkins University was first person to report the attempts of grafting pieces of lining of the amniotic sac on granulating wounds in 1910.

For the first time in 1913, Sabella reported the use of amniotic membrane on the raw surfaces caused by burn or ulceration. He applied the amniotic side of amniotic membrane towards the wound because of its ectodermal origin and observed reduced pain, rapid re-epithelization and absence of infection.

Brindeau (1935) and Burger (1937) used amnion for construction of artificial vagina. Burger successfully used the amnion also in repair of experimental dural defects in rabbits, dogs and cats. De Roth (1940) reported successful use of amnion in conjunctival repair.

Chao et al (1940) used the preparation of amniotic membrane known as " amnioplastin ". The amnion is imersed in alcohal for fixing, followed by drying in sheets and boiling in water for 20 minutes for sterilization. This was washed in normal saline before use. This fixed dead amnion preparation was used to prevent the adhesions following the craniotomy for head injury. He applied it over lacerated piamater; Dura mater and temporal muscle were stitched over it. There was no adhesions in any case. The amnioplastin gradually disappeared after 10 days. Mucoid material was present as remanent of membrane up to 20 days. There was slight evidence of mucoid material. On 30th day, there was no gross or microscopic evidence of mucoid material.

Histologically, after 10 days, there was no evidence of foreign body reaction or organized adhesions. The growth of fibroblasts beneath and above the membrane was only reaction to vanishing foreign body. Remains of amnioplastin was present as amorphus substance without cellular structure. After 30 days, dura and Pia defect was completely filled and there was no evidence of adhesion bands even over injured brain. Pinkerton (1942) reported the use of amnioplastin to prevent the adhesions between flexor tendons and their sheaths.

Kubani (1941-48) a Hungarian used amnion in various conditions like burn, traumatic skin loss, in prevention of

intra abdominal adhesions. He also tried it for repair of enterocutaneous fistula successfully in one case.

In 1950 Henson reported the use of amniotic membrane in the management of chronic skin ulceration and kept the smooth side of amnion for contact with wound surface. He found the granulation tissue never raised above margins as compared with other management of wound like coverage of wound under plaster of Paris.

Douglas (1952) reported the use of homografts of foetal membrane as a biological dressing over burn wound surfaces. In 1954, he used the human membranes on chorio-allantoic membrane of chick. He also studied the use of amnion and chorion as surface covering. Healing was quick and infection was less and he noticed that dressing separated readily from the surface, grafted with amnion, leaving a shiny, dry and pinkish surface. The chorion grafts w-ere more opaque and more salmon pink coloured.

By tissue chamber technique he observed that plasmic and haemic circulation remained active growing in perimeter of membrane transplant.

Jullian A. Sterling (1956) reported the successful use of the amniotic membrane over old infected flame burns. He suggested the amniotic membrane as dressing material for emergency management of trauma.

Hensen (1960) reviewed 100 cases of peripheral vascular disease treated by amnion implantation to the fat of thigh deeply. The patient were kept in bed for four days and normally discharged on 6th day without symptoms. Similar results were reported by Rowling (1958) under Hamilton (1958).

In 1960, Pigeon reported the application of amniotic membrane in burn cases with full aseptic precautions and observed following effects :

(A) Immediate effects :-

(1) Reduction in pain

(2) Antibiotics were used only in development of complication.

(3) Dressing were generally found quite dry.

(4) Healing of wound was rapid and complete.

(B) Delayed effects :-

(1) No discolouration

(2) Minimal scar tissue formation

He also stated that amniotic membrane undergoes change similar to which occur in cornified cells.

Massee and colleagues (1962) reported the use of foetal membrane to replace the parietal peritoneum in experimental studies in dogs undergoing pelvic exentration. Animals were killed after 59 days, there were very few adhesions and pelvic cavity was filled with dense scar tissue. Human trials were failed at that time.

Dino (1965) pointed out the use of amniotic membrane in burn cases. In favour of his study following points were suggested.

(1) It is the homograft which most closely resembles the skin being a direct continuation of foetal integuments along the umblical cord.

(2) It can be easily available and have minimal contact with the maternal blood.

(3) It is highly stretchable and can cover a wide surface.

(4) It is fairly strong to be handled.

(5) It is available with negligeable cost.

Dino (1966) preserved the amniotic membrane in following types of solutions.

(1) Sterile normal saline solution

(2) Densal koniom chloride (1:1000 dilution) in sterile saline solution.

(3) Sodium hypochloride (1:40 dilution) in sterile saline solution.

(4) Saline solution (400 cc) with 50,000 units of crystalline penicillin & 1 gm streptomycin sulphate.

(5) Saline solution (400 cc) with 1 gm Kenamycin sulphate.

The preserved grafts were kept in refrigerator at 4°c temperature. Bacteriological studies at regular interval were done to test efficacy of used preservative. Amniotic membrane

were preserved from fresh stage to one month and used in the treatment. From the bacteriological studies of preserved membrane which was done on Ist, 3rd, 7th, 14th and 30th day, he concluded that

(1) Solution of sodium hypochloride;

(2) solution of crystalline penicillin and streptomycin;

(3) kenamycin sulphate; were found to be best. On histological examination, cellular necrosis was seen from 2nd day after preservation. However according to kirschbain and hernaudaz (1963) cellular elements of amnion survived even after 45-60 days. Grossly the membrane remained intact and appeared thickened.

Galask et al (1970) observed the several antibacterial factors in amniotic membrane.

Trelfored and associates (1972) reported the preliminary results using amnion alone as an autograft and allograft in sheep. They reconfirmed, the Douglas observation that more consistent 'take' occurs if mesenchymal side was applied towards the host.

Robson et al (1973) on experimental rat burns, concluded that, compared to human skin, the amniotic membrane was more effective at decreasing the bacterial counts in the burn wounds. No specific antibacterial substance was found but it was proposed that the invitro antibacterial effect seen is due to achievement of a biologically closed wounds by the

membrane. Thus allowing the host's own defence mechanism to deal with the bacterial population. Similar observation was also found by Martin (1972) on experimental rats.

Robson et al (1973) treated 50 patients having open wounds with full thickness amniotic membrane. The foetal membrane was placed on the full thickness burn wound with the chorion against the granulating surface. The dressing was changed at every 48 hours. Specimens were taken for bacterial analysis at every change of dressing before and after. In partial thickness wounds, membrane were applied with chorion facing the wound and in some amnion facing the wound. They observed that foetal membrane adhered to all wound regardless of their depth. In all of the full thickness wound, the bacterial count decreased and the decrease was equal to allograft skin and superior to xenograft skin.

Tralfored and associates (1973) reported the use of amnion alone in full thickness fresh surgical wounds as bilogical dressing after radical volvectomies and groin dissections.

Colocho & others (1974) in the clinical and experimental studies, in 65 patients with split thickness donor sites, in 42 patients with partial thickness burns amniotic membrane was applied over the open wounds and in subcutaenous pockets in rats. He observed that if amnion was left exposed over partial thickness wound, it will dissecates and regeneration will begin by 3rd day and completed on 7th day of underlying epithelium of skin. No evidence of allergy,

rejection or neovascularization of amnion was noticed in any case. In experimental studies, amnion buried beneath the flap and continued to remain there, cellular integrity even after 5 days, suggesting the presence of plasmic and haemic circulation. None of the human donar site biopsy or India ink injection in experimental animals had shown communication with the host.

In 1974 Bapat and Kothari reported the successful use of living amniotic membrane graft for the restoration of the floor of the mouth in the patient of advanced cancer of the tongue following radical total glossectomy. Clinical observations showed that healing was rapid with induction of squamous metaplasia in 15 days without foul odour which is usually associated with skin grafts. Grafted area showed hardly any scarring. The floor remained flexible and pliable.

Marilyn trelfored - sauder and other (1977) reported the use of amniotic membrane to cover the raw area after pelvic extenteration, and its benefits: readily available tissue of low antigenicity, technically easy method for managing pelvic raw surface, reduced protein and fluid loss, reduced hospital stay and reduced intra-abdominal adhesions.

In 1978 they also reported the use of allograft amniotic membrane for control of intra abdominal adhesions.

Bose B (1979) reported the use of amniotic membrane over burn wound as biological dressing and stressed that

amniotic membrane adheres more firmly than other biological dressings. He recommended the use of amniotic membrane especially in developing country.

Mehta N. N. et al (1983) also recommended the use of human amniotic membrane as a biological dressing in burn wounds.

MATERIAL AND METHOD

MATERIAL AND METHODS

The present study has been conducted at M.L.B.Medical College and Hospital, Jhansi from June, 1983 to May, 1984 to evaluate the use of amniotic membrane as biological dressing in neglected cases of burn.

Collection of Amniotic membrane :

Placentae from clean vaginal deliveries and, emergency and elective caesarian section were collected by sterile technique. Placentae from mothers with history of premature rupture of membrane, veneral diseases, endometritis, pelvic inflammatory lesions, toxaemia of pregnancy and meconium staining or abnormal appearing liguor were rejected. The placentae with intact membrane was taken to directly in a clean tray and was washed thoroughly in running tap water to remove blood and mucoid material.

Separation of Amniotic membrane :

The thoroughly washed placentae was transferred to another clean tray filled with water. The amniotic membrane was separated from chorion and placentae, gently starting from the periphery up to the base of the umblical cord. The separated membrane was cut at the base of umblical cord and spread over flat surface in a sterile container filled with sterile normal saline. The remaining clots were removed from its surface with the help of sterilized gauge pieces. It is

washed further with sterile saline solution 3-4 times. Now the obtained amniotic membrane is thin, transparent, tensile, shining with whitish hue and strong which can cover a wide surface area.

Preservation of Amniotic Membrane :

The obtained membranes were either applied immediately or can be preserved in sterile normal saline (400 cc) treated with 10 lacs units of crystalline penicillin, 1 gm of streptomycin sulphate and 50 ml of liquid metrogyl; and kept at 4°c in refrigerator till the time of application. The preserved membrane was continously watched for bed odour, a change in colour from white to yellow or brown. Asepsis of membrane was tested by culture and sensitivity before application. The membrane showing negative culture can be used up to 8 weeks.

Selection of cases

All the cases with superficial and deep burns of less than 50% of body surface who came to the emergency or out patient department of this hospital after 72 hours of thermal injury were included in this study irrespective of their age, sex, Socioeconomic status, contamination of wound and mode of injury.

Method of study

The selected cases were subjected to detailed history and physical examination which were recorded on following lines:

i) History

Introduction :- Name, Age, Sex, Occupation, rural/urban, address, date of admission, date of discharge, time of healing and Number of repeated application of membranes.

- Date and time of burn (Duration of burn)
- Place of accident and nature of work at the time of accident.
- Cause of burn.
- Prior treatment (if any)
- Symptoms.

ii)Physical Examination

General Examination

General condition	-Pulse
Temperature	-Blood Pressure
Hydration	-Respiration

Local Examination

(A) Percentage of burn. It was calculated by 'Wallace's rule nine ' in the adult and by " Lund Browder Chart ", in children.

(B) Depth of burn : Superficial/deep

Estimation of Depth of burn

A hypodermic needle was used to test the pain sensation. The area with increased sensibility was considered to be superficial or partial thickness burn. The area with markedly reduced or absent pain sensibility was considered to be deep or full thickness burn. This was also confirmed by pulling out a hair from burn surface. In the 3rd degree

or deep burn, hair pulls out easily and painlessly. The later test is of value in borderline cases of 2nd degree burn. In addition help of following criteria was also sought.

Classification of depth	Appearance of burn area	Pain sensation
Ist degree	Erythematous	Painful and hyperaesthetic
IInd degree		
(A)	Blisteres with red dened base and moisture.	Painful and hyperaesthetic
(B)	Blisters with blanched base and moisture	Painful, hyperaesthetic or anaesthetic at places.
IIIrd degree	Leathery pale or pearly white or charred dry	Painless and anaesthetic

The I and II(A) were included as superficial and II(B) and III were considered as deep burn.

(C) Contamination of wound

Apparently clean : No contamination of foreign body, clean, intact blisters.

Mild contamination: Slight contamination, ruptured blisters, open wounds.

Gross contamination: Heavy contamination with dirty cloth, foreign body, dust and pus etc.

(D) Area involved: Diagramatic representation of area.

Resuscitation and General Management

The patients were resuscitated prior to application of membrane by I/V infusions, blood, plasma infusion, analgesic, antibiotics and tetanus prophylaxis.

LOCAL MANAGEMENT OF WOUND

Preparation of burn surface : A culture swab from burn surface was taken for culture and senstivity test. Patient was given necessary sedation or general analgesia after consent. A thorough debridement of wound was done by removing the necrosed skin, blisters and pus pockets. Then the wound was cleaned with 0.5% savlon solution three or four times and then final cleaning was done with normal saline twice. The spirit was applied over the adjacent skin around the margin of wound area. Any oozing from the wound area is stopped by pressure for some time.

Application of Amniotic membrane

Fresh or preserved amniotic membrane was stretched out and was applied on the burnt surface. The application was done in such a way that the membrane extend beyond the borders of the burn, overlapping the normal skin. This was done to keep the membrane in place as it adheres easily to dry skin. The amniotic membrane was applied with smooth surface facing the wound in case of superficial burn and glistening surface facing the wound in case of deep burn. All air and fluid blebs

were smoothened out to ensure total contact with the surface and excess membrane was trimmed. No dressing was applied over the part covered with the membrane. At least 6 hours bed rest was ensured to prevent dislodgement of the membrane.

In movable areas like the extremeties and joints, in uncooperative patients and children, the membrane was held in place by covering it first with sterile gauze then bandaging with sterile rolled gauze.

Assessment of the case

The assessment of the result was done daily following the application of the membrane.

The patients were asked about

1. Pain and discomfort prior and after application of the membrane.
2. Fever
3. Any evidence of allergy as itching rashes, nousea and vomiting.

Physical Examination

General Examination - Patients were examined for general condition, hydration, pulse, blood pressure and signs of toxaemia.

Local examination - Observation for the following was done -

1. Presence of discharge and/or soakage.
2. Appearance of amniotic membrane as regard to surface, margin, thickness, lusture, colour, dryness and adherence.

3. Collection of pus under dressing. If the pus was localized in small area underneath amniotic membrane puncture was done in it. A pus swab was taken for culture and sensitivity test. If the pus is underneath whole of membrane localized at many places, then the membrane was removed and reapplication was done after further cleaning and control of infection.

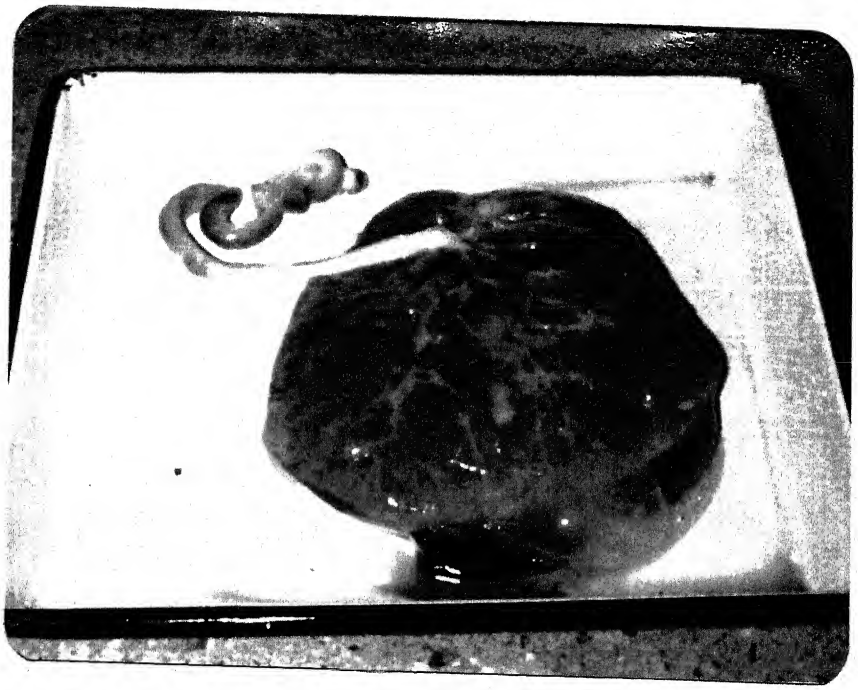
4. Result of healing.

Investigations

1. Routine - Blood - complete haemogram.
   Urine - Gross and microscopic examination.

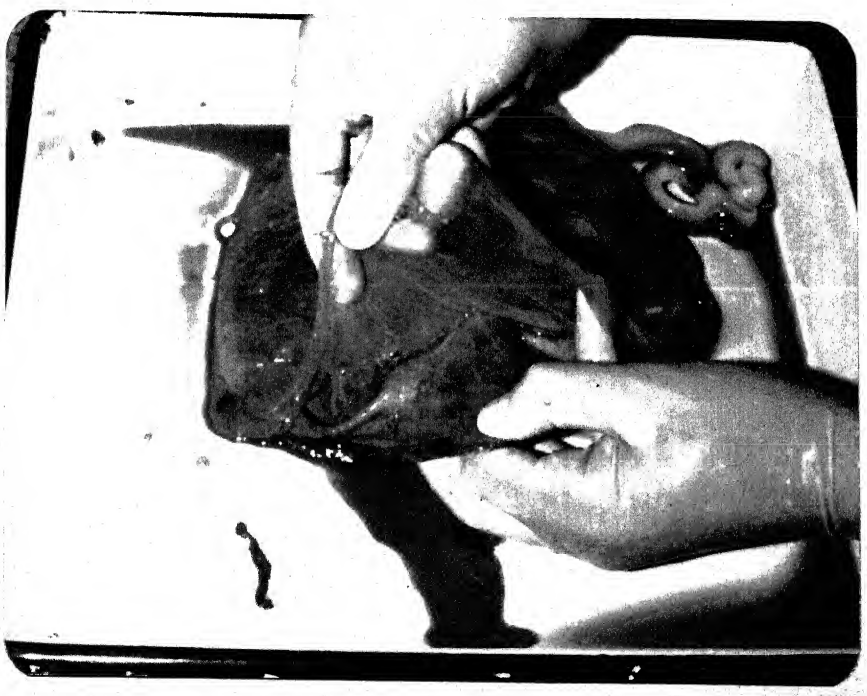
2. Culture and sensitivity test for pus if present.

This was cultured on blood agar and chocolate agar media which were kept in refrigerator at 0-4°c temperature for 24 hours. Antibiotic sensitivity was done in the cases where growth of pathogenic bacteria was revealed. Antibiotic was given according to the sensitivity reports.



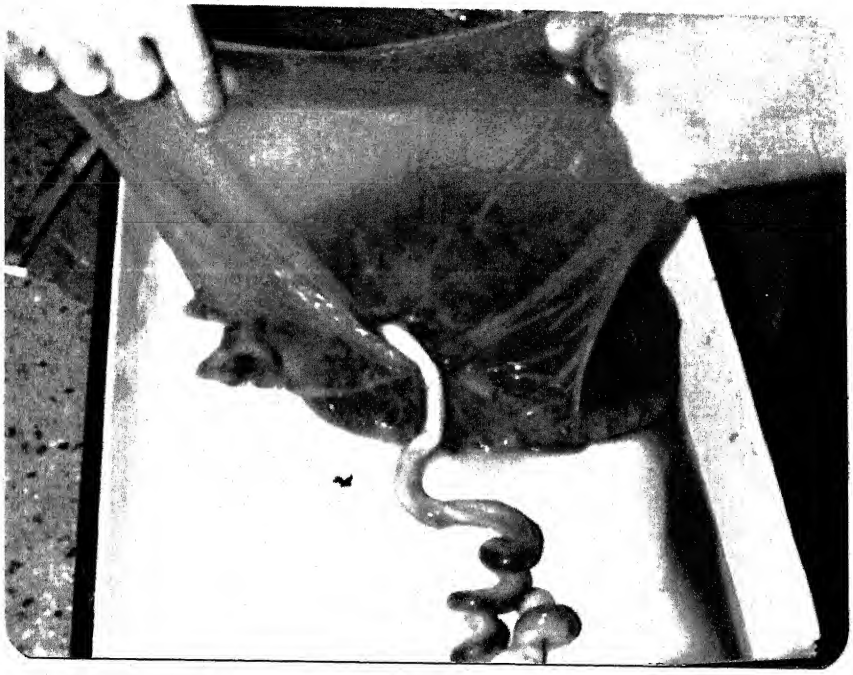
Photograph No. 1

Fresh Placenta in a tray after washing blood and mucoid material.



Photograph No. 2

Amniotic membrane is being separated from periphery.



Photograph No. 3

Amniotic membrane has been separated from periphery up to base of the cord.

Photograph No. 4

A preserved Amniotic Membrane.

PROFORMA

Name

Occupation

Address

Age/Sex

Rural/Urban

Date and time of admission

Date and time of discharge

Total time of healing

History

(i) Date and time of burn

(ii) Place of work and nature of work at the time of burn

(iii) Cause of burn

(iv) Prior treatment (if any)

Symptoms

(i) Pain

(ii) Burning

(iii) Blisters

(iv) Fever

(v) Discharge from wound surface

(vi) Any other

Physical Examination

a) General examination at the time of admission

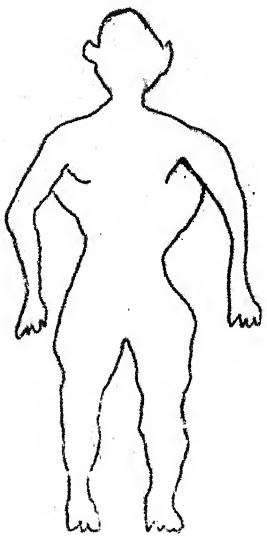
- G.C. -Pulse - B.P.

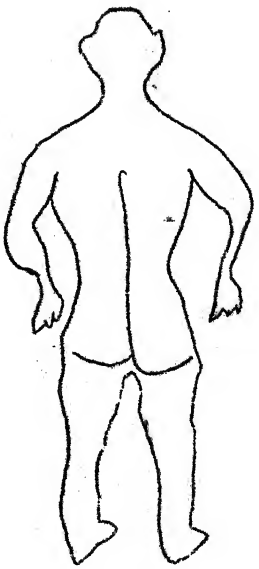
- Temperature -Respiration - Hydration

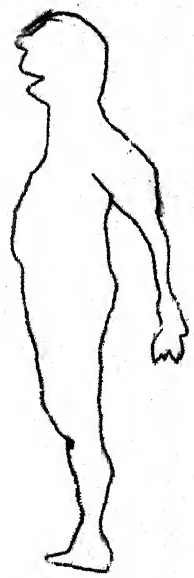
b) Local examination

- Percentage of burn
- Depth of burn/degree of burn
- Contamination
- Appearance of raw surface area
- Area involved (Diagramatic).

Anterior Posterior Lateral







Progress report

Days

G.C.

Pulse

B.P.

Hydration

Temperature

Differences between input output.

Albumin in urine

Days from membrane application	Number of membrane application Ist/2nd/3rd/4th..	Pain	Soakage	Biodressing changes					
				Surface	Marjin	Thicke-ning	Lustre	Colour	Dryness Adherenc
1st									
2nd									
3rd									
4th									
5th									
6th									
7th									
8th									
9th									
10th									
11th									
12th									
13th									
14th									
.									
.									
.									
.									

Time of healing and number of applications.

Investigations

Blood	- TLC	Urine	- Albumin
	DLC		Sugar
	Hb%		M/E
	ESR	Pus	- Culture & Sensitivity

Treatment

(i) I/V fluids

(ii) Blood

(iii) Sedatives

(iv) Analgesics

(v) Systemic antibiotics

(vi) Local application

OBSERVATIONS

OBSERVATIONS

The present study consists of 30 patients of neglected burn admitted in surgical and emergency wards and also as O.P.D. cases in Surgery at M.L.B. Medical College, Jhansi, from June 1983 to May 1984. These patients belonged to different social strata and were of either sex. These patients had age group ranging from 3 years to 35 years and had burns involving less than 50% of total body surface. Only those cases were included on whom amniotic membrane was applied after 72 hours of having sustained burn injuries.

Of the total 30 patients, 28 patients were below 30 years of age.8 cases (6 male and 2 females) ie. 26.667% were below 10 years of age, 9 cases (2 males and 7 females) ie. 30% between 11-20 years of age and 11 cases (6 males and 5 females) ie. 36.667% were between 21-30 years of age. Only two females patients were between the age group of 31-40 years. Total male patients were 14 (46.667%) and female patients 16 (53.333%).

TABLE NO. 1

Showing Age incidence

Sl.No.	Age group	Number of cases	Percentage
1	0-10	8	26.667
2	11-20	9	30.007
3	21-30	11	36.667
4	31-40	2	6.667
5	41-50	-	-
Total		30	100

TABLE NO. 2

Showing sex incidence in different age groups

Sl. No.	Age group (Years)	Male		Female	
		No.	%age	No.	%age
1	0-10	6	20	2	6.667
2	11-20	2	6.667	7	23.333
3	21-30	6	20	5	16.667
4	31-40	-	-	2	6.667
5	41-50	-	-	-	-
Total		14	46.667	16	53.333

Maximum burn injuries 27 (11 males and 16 females) ie. 90% occured at home when the patients were engaged in their daily home tasks. 3 patients (10%) sustained burns while working in out door all of these were males.

TABLE NO. 3

Showing location of burn accident

Sl. No.	Location	Male		Female	
		No.	%age	No.	%age
1	Indoor	11	36.667	16	53.333
2	Outdoor	3	10.000	-	-
Total		14	46.667	16	53.333

21 (70%) out of 30 patients hailed from rural area and 9 (30%) from urban.

TABLE NO. 4

Showing Rural/Urban incidence

Sl.No.	Rural/Urban	Male	Female	Total	%age
1	Rural	10	11	21	70
2	Urban	4	5	9	30
Total		14	16	30	100

It was observed that 13 patients (4 males and 9 females, ie. 43.333% sustained burn injuries from fire while cooking food or working near the fire. 5 patients (2 males and 3 females) ie. 16.667% had burns due to lamp fire. 6 patients (4 males and 2 females) ie. 20% suffered burn injuries due to scalding, 2 male patients suffered injuries while working on electric installation, 4 patients (2 males and 2 females) suffered burn injuries due to touch with hot metal or cracker. 11 cases were superficial, 5 cases were deep and 14 cases were mixed.

Of the 30 patients, 11 were housewives, 10 student 3 labourers, 3 Government servant and 3 were children.

TABLE NO. 5

Showing causes of burn according to sex

Sl.No.	Causes	Male	Female	Total	%age
1	Fire during work or cooking	4	9	13	43.333
2	Lamp	2	3	5	16.667
3	Scalding	4	2	6	20.400
4	Electric current	2	-	2	6.667
5	Miscellaneous	2	2	4	13.333
Total		14	16	30	100

TABLE NO. 6

Showing different occupation among burn patient

Sl.No.	Occupation	Number of cases	Percentage
1	Housewives	11	36.667
2	Labourer	3	10.000
3	Student	10	33.333
4	Government servants	3	10.000
5	Miscellaneous	3	10.000
Total		30	100

16 patients ie. 53.333% out of 30 reached hospital within 5 days of injury. 8 patients (26.667%) reached between 6 to 10 days, 2 patient (6.667%) between 11-15 days and 2 patients (6.667%) reached between 16 to 20 days after sustaining burn injuries. Two patients (6.667%) attended hospital after six and seven months of burn.

Off all these cases, 17 (56.667%) were grossly contaminated and 13 (43.333%) presented with mild contamination. Two cases attended hospital after few months of burn injuries were having burn contracture also.

TABLE NO. 7

Showing time interval between burn accident and hospital arrival

Sl.No.	Duration in days	Number of cases	Percentage
1	0-5	16	53.333
2	6-10	8	26.667
3	11-15	2	6.667
4	16-20	2	6.667
5	20- more	2	6.667
Total		30	100

TABLE NO. 8

Showing the Grade of contamination

Sl.No.	Grade of contamination	No.of cases	%age
1	Mild contamination	13	43.333
2	Gross contamination	17	56.667

Out of 30 cases where membrane applied, 14 cases (46.667%) were having less than 10% burns, 6 cases 11-20%, 5 cases 21-30% and 5 cases were having 31-40% of burns.

TABLE NO. 9

Showing percentage of burn

Sl.No.	Percentage of burn	No.of cases	%age
1	0-10	14	46.667
2	11-20	6	20
3	21-30	5	16.667
4	31-40	5	16.667
5	41-50	-	-

In 12 cases (40%) membrane were applied within 5 days of burn injury, in 10 within 6 to 10 days, in 1 patient between 11 to 15 days, in 3 patients between 16 to 20 days, in 2 patients between 21 to 35 days and in 2 patients membrane were applied after more than 25 days.

TABLE NO. 10

Showing time interval between burn accident and application of membrane.

Sl.No.	Time of interval in days	No.of cases	%age
1	0-5 days	12	40
2	6-10days	10	33.333
3	11-15days	1	3.33
4	16-20days	3	10.000
5	21-25days	2	6.667
6	26-more	2	6.667
Total		30	100

Out of 30 cases, 21 patients applied some local preparation over burn area, 8 patients applied nothing and in one patient membrane was applied just after burn that had become infected. On culture and sensitivity of the discharge from the burn areas, 17 cases were having growth of pseudomonas 2 cases were positive for the growth of staphylococcus aureus, and 2 cases were showing growth of staphylococcus pyogenes . One case showing growth of Klebsiella also and 9 cases were not showing any growth of bacteria.

TABLE NO. 11

Showing prior treatment in burn areas

Sl.No.		No.of cases	%age
1	Local application	21	70
2	Amniotic membrane application	1	3.333
3	No prior treatment	8	26.667
Total		30	100

TABLE NO. 12

Showing Growth of bacteria over burn area

Sl.No.	Bacteria growing over burn area	No.of cases
1	Pseudomonas	17
2	Staphylococcus aureus	2
3	Staphylococcus pyogenes	2
4	Klebsiella	1
5	No growth	9

After application of amniotic membrane, following main criteria were considered viz. relief of pain and discomfort, development of fever and allergic reactions, control of oozing and gross infection. The number of repeated application, total duration of healing and condition of wound after separation of membrane were observed.

In all 30 amniotic membrane treated wounds relief of pain and discomfort was recorded and no analgesic was required even though sedatives were required only to relieve the anxiety of the patient. No allergic reaction was seen in any case. Fever was recorded in patients when pus formed under the membrane and that subsided after next application of membrane with support of sensitive antibiotic.

In 26 cases, oozing stopped within 24 hours of application of amniotic membrane. In other four cases which were very deep, oozing stopped within 24-36 hours.

It was observed that all wounds, treated by amniotic membrane became dry and adherent to the wound surface within 6 to 12 hours of application in summer season and in winter and humid atmosphere within 12-24 hours of membrane application.

In about 24-48 hours, membrane covering the adjacent normal healthy skin started curling up but remained adherent to wound surface.

In cases where no soakage occured under membrane, the membrane became opaque and dried with its colour changing from yellowish white to light brown. If pus formed under membrane within 2 or 3 days the colour of membrane changed to yellow at sites and looked wet. If pus formed after 2-3 days where membrane had already dried, only separation of membrane occured from the wound area with discharge of pus from the margin. In 4 cases, the pus was removed, immediately as it was seen under the membrane, by puncturing the membrane at the site of collection of pus. These cases were superficial and mixed deep burns of within 15% of body area. In 2 cases of these the contamination was mild and in other 2 it was gross.

Supportive antibiotic was started based on culture and sensitivity report. These cases did not need second application of membrane and healed within 10 to 18 days of membrane application.

TABLE NO. 13

Showing number of application of membrane for percentage of burn

Sl. No.	Percentage of burn	No. of cases with only one application	No. of cases with two application	No. of cases with three application	No.of cases with four application	Total
1	0-5	6	-	-	-	6
2	6-10	7	-	-	1	8
3	11-15	1	-	1	1	3
4	16-20	1	1	-	1	3
5	21-25	1	-	-	-	1
6	26-30	1	1	-	2	4
7	31-35	-	-	-	-	-
8	36-40	1	-	-	4	5
Total		18	2	1	9	30

TABLE NO. 14

Showing relationship between total healing time to contamination of wound and depth of burn.

Sl. No.	Total healing time in days	No.of cases	No.of cases with mild contamination	No.of cases with gross contamination	Depth of burn		
					Sup.	Deep	Mixed
1	0-10	1	1	-	1	-	-
2	11-20	13	8	5	9	2	2
3	21-30	5	3	2	1	-	4
4	31-40	5	1	4	-	-	5
5	41-50	1	-	1	-	-	1
6	51-60	3	-	3	-	2	1
7	Not healed	2	-	2	-	2	-
Total		30	13	17	11	6	13

The varying number of repeated application of membrane were needed for different percentage of burn. All the 6 cases of 0-5% burn needed only one application of membrane. Out of 8 cases of 6-10% burn, 7 cases needed one application and 1 case needed as many as 4 applications but did not heal due to contracture. Out of 3 cases of 11-15% of burn, one case needed on application, 1 case needed three applications and 1 case needed four applications. Out of 3 cases of 16-20% burn, 1 needed one application, 1 needed two applications and 1 needed four applications of membrane for complete healing of burn wound area. 1 case between 21-25% of burn needed only one application. In 4 cases of 26-30% of burn, 1 case needed one application, 1 case needed two applications and 2 cases needed four applications of membrane for total healing. Out of 5 cases of 36-40% of burn, 1 case needed only one application and 4 cases needed four applications in which one case did not heal due to sarrounding contractures already present.

The rate of healing was also observed in relation to contamination of wound at the time of application and depth of burn. 1 case which healed in 10 days was having mild contamination over superficial burn. Out of 13 cases, healed between 11-20 days, 8 cases were having mild contamination and 5 cases had gross contamination. In these cases, 9 were superficial, 2 were deep and 2 were of mixed depth of burn. In 5 cases which healed in 21-30 days, 3 were having mild contami-

nation and 2 were having gross contamination. Out of 5 cases healed in 31-40 days, 1 case was having mild contamination and 4 cases were having gross contamination. All were of mixed depth of burn. One case of mixed depth of burn with gross contamination healed in 41-50 days, 3 cases with gross contamination healed in 51-60 days. Out of 3, two were purely deep burn and 1 was of mixed type. Two cases with gross contamination and sarrounding contracture did not heal.

TABLE NO. 15

Showing relationship between healing time and interval between burn injury and membrane application

Sl. No.	Healing time	Interval between burn injuries & membrane application						
		3-7	8-12	13-17	18-22	23-27	7/28	Total
1	10 days	-	-1	-	-	-	-	1
2	11-20 days	9	3	-	-	1	-	13
3	21-30 days	3	1	1	-	-	-	5
4	31-40 days	1	2	1	1	-	-	5
5	41-50 days	1	-	-	-	-	-	1
6	51-60 days	1	-	1	1	-	-	3
7	Not healed	-	-	-	-	-	2	2
Total		15	7	3	2	1	2	30

TABLE NO. 16

Showing healing time in relation with percentage of burn

Sl. No.	Healing time in days	Number of cases in different %age of burn				
		0-10%	11-20%	21-30%	31-40%	Total
1	10 days	1	-	-	-	1
2	11-20 days	10	2	1	-	13
3	21-30 days	2	-	2	1	5
4	31-40 days	-	2	2	1	5
5	41-50 days	-	-	1	-	1
6	51-60 days	-	1	-	2	3
7	Not healed	1	-	-	1	2
Total		14	5	6	5	30

The rate of healing was observed in relation to percentage. 1 case which healed in 10 days was having superficial burn between 0-10%.out of 13 cases, healed in 11-20 days, 10 cases were of 0-10% burn and 2 cases of 11-20% age and 1 case was having burn between 21-30% age. In 5 cases healed in between 21-30 days, 2 cases were having 0-10% of burn, 2 cases 21-30% and one case was having 31-40% of burn. Out of 5 cases of burn, healed in 31-40 days, 2 were having 11-20% burn, 2 were 21-30% and 1 was having 31-40% of burn. One case which healed in 41-50 days was having 21-30% of burn. Out of 3 cases, healed in between 51-60 days, 1 case was having 11-20% of burn and remaining two cases were having 31-40% of burn. Two cases

did not heal. One was having 11-20% of burn and other 31-40% of burn, but both had contracture already established after burn.

The rate of healing was also observed in relation to interval between burn injury and membrane application. One case in which membrane was applied between 8-12 days of burn injury, healed in 10 days. Out of 13 cases, healed in 11-20 days, membrane was applied between 3-7 days of burn injury in 9 cases, between 8-12 days in 3 cases and between 23-27 days in 1 case. Out of total 5 cases which healed between 21-30 days, membrane was applied between 3-7 days of burn in 3 cases, between 8-12 days in 1 case and between 13-17 days in 1 case. 5 cases, healed in between 31-40 days, in which membrane was applied between 3-7 days in 1 case, between 8-12 days in 2 case, between 13-17 days in 1 case and between 18-22 days of burn injury in 1 case. One case in which membrane was applied between 3-7 days of burn injury healed in between 41-50 days. Out of 3 cases, healed in between 51-60 days, membrane was applied in between 3-7 days after burn injury in 1 case, 13-17 days, after burn in 1 case and between 18-22 days after burn in 1 case. In two cases where membrane was applied after 6 months, no healing was seen even after four applications. Both these patients were having contracture around the burn wound and they were subjected later on for surgery in form of release of contracture and skin grafting.

TABLE NO. 17

Number of repeated applications of membrane needed in relation to time interval between burn & first membrane application

Sl. No.	Time interval between burn & membrane application	No. of cases healed with one application	No. of cases healed with two application	No. of cases healed with three application	No. of cases healed with four application	Total
1	3-7 days	11	2	-	2	15
2	8-12 days	5	-	-	2	7
3	13-17 days	1	-	-	2	3
4	18-22 days	-	-	1	1	2
5	23-27 days	1	-	-	-	1
6	27-more	-	-	-	2	2

The number of repeated application of membrane required for healing was also observed with respect to time interval between burn accident and first membrane application. Out of 15 cases where membrane was applied after 3-7 days of burn accident, 11 cases needed only one application for total healing, 2 cases needed two application and two cases needed four application of membrane. In 7 cases where membrane was applied between 8-12 days after burn, 5 cases needed application of membrane once and two cases needed four times application of membrane. Out of 3 cases in whom membrane was applied for the first time after 13-17 days of burn, 1 case needed one application and other two cases needed

area.

In two cases where membrane application was started after 18-22 days of burn, one case needed three applications and other case needed four applications of membrane for complete healing. One case needed only one application of membrane which was applied after 23-27 days of burn. In two cases where membrane was applied after six months of burn accident four time application of membrane was done, but no signs of healing was seen. So the cases were treated further by skin grafting after contracture release.

Not a single case, which healed after repeated application of membranes, developed keloid or contracture.

Photograph No. 5-A

A Neglected case of burn 8 days old.

Photograph No. 5-B

The application of amniotic membrane after cleaning in the above patient.

Photograph No. 5-C

The same patient after 13 days of application of amniotic membrane showing complete healing.

Photograph No. 6-A

The patient showing infected burn of 7 days old.

Photograph No. 6-B

The cleaned raw area in the same patient.

Photograph No. 6-C

Showing immediate applied amniotic membrane over cleaned area in the same patient.

Photograph No. 6-D

Showing progressive healing from the periphery -on 20th day of membrane

Photograph No. 6-E

Showing complete healing after 30th day of membrane application.

Photograph No. 7-A

A patient of infected burn after 19 days of amniotic membrane application.

Photograph No. 7-B

Showing burn area after cleaning.

Photograph No. 7-C

Showing amniotic membrane over burn area just after application.

Photograph No. 7-D

Showing autolysis of amniotic membrane with pus under neath after 5th day of application.

Photograph No. 7-E

Showing burn area after cleaning in the same patient.

Photograph No. 7-F

Showing second application of amniotic membrane.

Photograph No. 7-G

Curling of Amniotic membrane from periphery - After 3 days of application.

Photograph No. 7-H

Showing healing at periphery and autolysis of membrane in the centre.

Photograph No. 7-I

Showing progressive peripheral healing and central autolysis of membrane after 3rd application of the membrane in the same patient.

DISCUSSION

D I S C U S S I O N

Burn management is still in the phase of trial. After so many advances in medical sciences mortality from burn has definitely reduced but morbidity from contracture and keloid are still crippling the sufferer physically, mentally as well as socially.

Burns give rise to raw areas which are prone for invasion by micro organisms and abnormal loss of body constituents in the form of water, minerals and proteins. The loss of blood flow which occurs immediately after burn, starts to return slowly after 24 hours as patent vessels reappear. The delicate dynamic process of revascularization for the purpose of repair is associated with local circulatory stasis which causes vulnerability of wound to dessication and infection. Either of these can trip the balance converting it into a zone of necrosis or full thickness burn.

The water retention ability of skin depends on its effective vapour pressure and diffusion barrier offered by keratin layer and lipid content in the stratum cornium. This lipid is thermolabile and is easily destroyed by heat. If the lipid barrier is removed by thermal injury, the effective vapour pressure gradient is increased by 15-20 times (normal $1.5 \pm .08$ mm above atmospheric pressure). This results into a

large amount of evoporative water loss amounting to 3-10 times of normal rate insensible water loss of 40 ml per hour. The amount and duration of loss depends on depth of burn.

Therefore the main emphasis in the treatment of burn is to re-establish the continuity of skin. The raw area after burn should be covered to make it a closed wound which subsequently reduces excessive evoporative water loss and prevents wound infection.

The autografts are the best to cover the raw area but these have their limitations. Alternatively homografts are used but here again availability is limited. Other biological covering materials are allograft skin, heterografts skin, collagen sheet, foetal membranes etc. The cadaver skin is in limited supply in general hospital and it is expensive also.

The concept of temporary biological dressings was introduced in 1930 by Brown. Homografts and heterografts split thickness skin have both proved to serve the functions required of a biological dressing. In 1953, Brown et al reported that it was practical to use postmortem homografts as biological dressings. Since then, cadavers have provided the usual source for homografts. Following the recommendations of silvitti et al heterografts were introduced to eliminate

the problem of avialability of homografts. Heterografts have not proved as effective as homografts in decreasing bacterial contamination of the wounds.

Amniotic membranes were chosen for evaluation. It is not necessary to point out how easy and impersonal it is to obtain this widely distributed human material which at present, seem to find its only destiny to be " Thrown into the bucket ", especially if it is normal. It has been stated that since amniotic membrane is formed by the ectoderm of the foetus, it is like an extension of the body skin.

Amnion, chorion and the combined foetal membrane have been used by various investigators as a substitute for skin in the past. Since sabella's first case describing the use of amniotic membrane in the burn wound 50 years ago, multiple reports have appeared in the world's literature. Most of these were reporting the attempts to use amniotic membrane as a permanent substitute for skin autografts or as a dressing over partial thickness burns. Dahinteroa and Dobrkovsky observed failure when amniotic membranes were applied in deep burns or on severely infected areas. They pointed out that the membranes became autolyzed in 48 hours and disintegrated. Furthermore they stated that the same was true on all granulating surfaces even if they were clean. Similar findings have been reported

by others. In these cases, the membranes were changed every 48 hours. As demonstrated by Shuck and Mancrief for homograft skin, in a less tidy wound, more frequent changes prevent collection of purulent material under the biological dressings. This allows firm adherence of the membrane to the underlying granulation.

Frequently changed amniotic membrane were more successful in decreasing the bacterial count in contaminated rat burns than human skin. This raised the question as to whether there was a substance in amniotic membrane which was specifically antibacterial. One such possibility is allantoin which is known to exist in amniotic membrane. Another possibility is lysozymes, a bacteriolytic protein of low molecular weight which is present in amniotic tissue. Rubin and Bargiovi recently stated that skin itself possesses bactericidal substances in its biological make up such as lysozymes and certain fatty acids. Neither, however, could they demonstrate bacterial inhibitory activity of split thickness human skin in vitro when measured by a disc sensitivity technique.

Another hypothesis for the observed decrease in the bacterial count under the amniotic membrane lies in the intimate biologic closure of the open wound by the membrane. Restoration of the functional circulation through the covered granulations allows a more rapid turn over of phagocytes, serum bacteriolytic factors and may accelerate the removal of

necrotic debris. Therefore repeated application of the membranes, allows the host resistance factor in the granulating bed to function at peak efficienty. The increased antibacterial effects seen with the amniotic membrane may be due to the fact that it is less well differentiated than skin.

The amniotic membrane fulfilled all of the functions of an ideal biological dressing. In terms of their large size and ready availability at no cost to the patient, they are actually superior to homograft and heterograft skin. In addition, the membranes appear to have another property subjectively, the rapidity of ingrowth of epithelium from the borders of the wound in full thickness defects and the rate of reepithelization of partial thickness burns appear to be increased by their use. Chao et al and Troensegaard-Hansen also have noted that amniotic membrane seemed to posses some specific healing power. They have reported a stimulation of both fibrous tissue growth and more rapid epithelial repair.

The amniotic membrane after collection from the labour room can be preserved in different types of preservatives. Selection of the preservative is based on reports of Dino (1966). From bacteriological study of preserved amniotic membrane, he concluded that : 1 solution of sodium hypochlorite 1:40 dilution in sterile saline, 2. Sterile

saline solution (400 ml) with 1 gm of kenamycin sulphate, 3. Saline solution (400 ml) with 10 lacs unit of crystalline penicillin and 1 gm streptomycin sulphate were found to be best for preservation of the membrane upto 30 days. In the present study, 50 ml of metrogyl was also added to saline solution mixed with crystalline penicillin and streptomycin. On bacteriological reports, it was found that addition of metrogyl has extended the time of preservation upto 60 days, but the biological property of membrane reduced in such a long time. It is seen that the biological property gradually decreases with the time.

In the present study, it has been noted that pain and discomfort disappears, immediately after application of amniotic membrane and no further analgesics or sedatives were required after the dressing. Occasionally sedative was required for psychological support. No allergic symptoms like rigor, rash, vomiting and giddiness were noticed even after close watch. These findings are comparable with the published reports of other workers. The cause of disappearance of pain and discomfort is coverage of exposed nerve endings.

It has been observed that amniotic membrane adhered and became dry in 6-8 hours in hot and dry atmosphere and in 12-24 hours in cold and wet atmosphere. Adherence has been

proposed to be most important property of biological and synthetic materials applied to deepithelized surfaces. It reduces pain, bacterial contamination and consequently optimize the rate of healing. Most prosthesis and grafts rely on the endogenous adhesive fibrin for adherence. This property of material is therefore determined by the strength of bond that it forms with fibrin. Studies have demonstrated that fibrin bonds preferably to collagen in normal skin.

In most of superficial burn cases with mild contamination, where membrane was applied after proper cleaning no soakage was seen. Cases needed only one application and healed quickly.

It has been observed that out of 30 amniotic membrane treated wounds 13 wounds (43.33%) healed within 11-20 days, 5 wounds (16.67%) healed in between 21-30 days 5 wounds (16.67%) healed in between 31-40 days 6 wounds (3.33%) in 41-50 days, 3 wound (10%) in 51-60 days and 1 wound (3.33%) healed in 10 days. 2 wounds (6.67%) did not heal at all after four application of amniotic membrane. Most of those cases which healed between 11-20 days needed only one application of membrane. Two of the cases did not heal at all, or have not shown any sign of reepithelization. These cases were having contracture around the wound area and

they were near the joint. It has been thought that the contracture and its sarrounding severe fibrosis have not allowed the contraction of wound area or epithelial proliferation.

On observation at rate of healing in relation to percentage of burn, It has been seen that burn with less percentage healed early in comparison to large percentage. 10 cases (33.33%) healed within 11-20 days, were having only 0-10% of burn. 2 cases (6.66%) which healed within 51-60 days were having 31-40% of burn.

Rate of healing also depends on the contamination of wound at the time of membrane application. Out of 30 cases, 13 cases (43.33%) were having mild contamination and 17 cases (56.67%) were having gross contamination. This contamination was confirmed by sending pus swab for culture. It has been seen that pseudomonas was positive in 17 (56.67%) pus cultures, Staphylococcus aureus in 2 (6.67%) pus culture, Staphylococcus pyogenes in 2 and klebsiella was positive in 1 pus culture. After cleaning the wound area membrane was applied with systemic antibiotic support. In most of the cases the bacterial senstivity was for Gentacyn and Ampicillin. At every reapplication, pus culture was sent and antibiotic was changed accordingly. Out of 13 cases with mild contamination, 8 cases healed within 11-20 days, 3 cases within 21-30 days and 1 case healed within 10 days of

membrane application. Out of 17 cases with gross bacterial contamination most of cases healed between 20-60 days and needed more than one amniotic membrane applications. This gives the idea that bacterial contamination delays healing but amniotic membrane has definitely checked the growth of bacteria over the wound area. Julean, A sterling (1950) used the amniotic membrane to treat the old infected flame burns and reported successful results. Martin (1972) in his experimental study on infected wounds of rats concluded that amniotic membrane has been shown to control the bacterial growth as isograft skin. Exact mechanism of reduction in bacterial level of wound is unknown. But different workers have suggested different mechanisms. Golosk and Snyder (1970) have demonstrated the presence of multiple factors in human amniotic fluid. Those are said to be antibacterial. Chalacho et al (1974) stated that whether those antibacterial factors present in the amniotic fluid are retained in amniotic membrane or not, is uncertain. Should they be present, value of this material for dressing may be enhanced. Martin (1972) using in vitro technique stated that no antibacterial substance could be found which were sought previously. He proposed that in vivo antibacterial effect seen is due to achievement of biological closed wound by the membrane and this allows the host's own defence mechanism to deal with bacterial population as did other biological dressings.

Adherence of skin substitutes is also said to be reducing the bacterial infection by some workers, in split thickness burns and donar sites.

The contamination is directly proportionate to the neglect of wound. Out of 30 cases, 21 cases applied some local preparations, 8 cases applied nothing over wound area and in 1 case membrane was applied immediately after burn. After application of membrane, that case has been discharged from hospital without support of antibiotic and patient has not taken any precaution at home to avoid contamination. The degree of contamination increased with the time interval between burn accident and membrane application. Most of these cases came to the hospital after 3 to 20 days of burn and healed within 10 to 60 days. These cases needed repeated application of membrane, maximum upto four applications. The number of application required, depended upon the depth of burn and contamination. The less the depth of burn and contamination, the less time and number of applications needed for complete healing. The contamination increases with the time of neglect of burn. This pyogenic infection increases the depth of burn and causes early autolysis of membrane, requiring more number of membrane application for healing. This observation has already been reported by Dehinterova & Dobrokovsky.

Two cases came in the hospital after 6-7 months of burn injury. These were the cases where contractures had developed and the sarrounding unhealthy scar prevented epithelization of the remaining raw area. Here the granulation tissue was very well developed and probably autolysis of the membrane occured every time it was applied (4 times) resulting into complete failure of the membrane application.

It has been observed that cases needing repeated application take too much time in healing as in cases ordinarily being dressed with ointments etc. Still economically, the membrane application treatment remains cheap and also formation of the keloid & contracture is much less.

CONCLUSION

CONCLUSION

The effect of amniotic membrane was studied in 30 cases of neglected burns involving less than 50% of body surface area. The following conclusions were down.

1. Females are little more sufferer than males for they are exposed to danger due to house work.
2. The incidence of burn is much higher in younger age group i.e. below 30 years of age.
3. Most of the burns are thermal in nature.
4. Most of the burns occurred during indoor activity.
5. Amniotic membrane provides good coverage to raw area.
6. Amniotic membranes are easily collected and preserved which can be used safely several days after preservation without changing their biological nature.
7. After membrane application, the open wound changes into biological closed wound thus preventing protein and fluid losses from the raw surface, at the same time they prevent further infection from outside.
8. It helps to prevent conversion of superficial burns to deep burns, thus promoting healing.
9. The discomfort and sufferings of the patient is immediately removed after membrane application.
10. The sufferings from daily dressing is prevented.

11. Repeated application of membrane in contaminated wound healed normally without formation of keloid or contracture which are usual sequelae to deep burns with treatment by open dressing.
12. The quality of healed wound are pink, smooth and flat margins.
13. The only unsuccess was seen that the time taken in healing of neglected cases of burn with repeated applications of membrane is more or less same as in ordinary daily dressings.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Adams,F(1939): quoted by Hauben, D.J.; Yanai,E.; Mahler,D: On the history of the treatment of burns. Burn,7:383,1981.

2. Aegineta,F(1538): quoted by Hauben,D.J.; Yanai,E.; Mahler,D: On the history of the treatment of burns. Burn,7:383,1981.

3. Agarwal,V.K.: Uses of amniotic membrane as biological dressing in superficial burn. A thesis for M.S.Surgery, Bundelkhand University, Jhansi, 1982.

4. Artz,C.P.: Management of thermal burns. Mod. Med. 26:181,1960.

5. Bapat,C.V.; Kothari,P: Preliminary report on acceleration of wound healing by amnion grafts. Ind.J.Med.Res., 62:9,1974.

6. Baxter,C.R.: Homografts and hetrografts as a biologic dressing in the treatment of thermal injury. Presented at the first annual congress of the Society of German Plastic Surgeons in Munich, Germany, September 28,1970.

7.a. Blalock,A.: Experimental shock: Possible causes for reduction in blood pressure, following mild trauma to extremity. Arch Surg., 22:598,1931.

7.b. Blalock,A.: Experimental shock: composition of fluid that escapes from blood stream after mild trauma to extremity, after trauma to intestines and after burns. Arch.Surg., 22:617,1931.

7.c. Blalock,A.: Experimental shock: the importance of local loss of fluid in the production of low blood pressure after burns. Arch. Surg. 22:610,1931.

8. Bose,B.: Burn wound dressing with human amniotic membrane. Ann. R. Coll.Surg. Engl., 61(6):444,1979.

9. Boyer,A.(1814):quoted by Hauben,D.J.; Yanai,E.; Mahler,D: On the history of the treatment of burns. Burn,7:383,1981.

10. Brown,W.H.(1896); quoted by Hauben,D.J.; Yanai, E.; Mahler,D: On the history of the treatment of burns. Burn,7:383,1981.

11. Brown,J.B. and Mc,Dowell: Massive reports of burns with thick split thickness grafts. Ann. Surg, 115:658,1942.

12. Brown, J.B. and Frayer, M.P.: Post mortem homografts to reduce mortality in extensive burns. J.A.M.A., 165:1163,1954.

13. Burger,K:Experimental and clinical studies on transplantation of foetal membrane, Orv Hetil, 82:800,1937.

14. Burleson,R. and Eisman,B: Nature of the bond between partial thickness skin and wound granulations. Surgery, 72:315,1972.

15.a. Burleson,R. and Eisman,B: Mechanism of antibacterial effect of biological dressings. Ann. Surg., 177:181,1973.

15.b. Burleson,R. and Eisman,B: Effect of skin dressings and topical antibiotics on healing of partial thickness skin wounds in rats. Surg. Gynaec. Obstet, 136:958,1973.

16. Chao, Y.C.; Humphrey,S.S. and Penfield,W: A new method of preventing adhesions: The use of amnioplastin after craniotomy. Brit. Med. J., 1:517,1940.

17. Clowes,W.(1591):quoted by Hauben,D.J.; Yanai,E.and Mahler,D: On the history of the treatment of burns. Burn, 7:383,1981.

18. Colocho,G.; Grahm,W.P. and Green,A.E.: Human amniotic membrane as a physiological wound dressing. Arch. Surg. 109:370,1974.

19. Copeland,W.P.(1887): quoted by Hauben,D.J.; Yanai,E.; Mahler,D: On the history of treatment of burns. Burn, 7:383,1981.

20. Dago,G.: Survival and utilization of cadaver skin. Plast Reconst. Surg., 10:10,1952.

21. Davis,J.S.: Skin transplantation with a review of 550 cases at the John Hopkins Hospital. J.H.H.report,15:307,1910.

22. Davix, J.S.: Plastic Surgery: Its principles and Practice P. Blakistons son & co. Philadelphia. P. 1919,1919.

23. Dahinterova,J. and Dobrokovsky, M.: Treatment of the burned surface by amnion and chorion grafts. Sbon. Ved. Prac. Lek. Eak. Karlov. Univ. Suppt.,51:513,1968.

24. Davidson,E.C.: Tannic acid in the treatment of burns surg. Gynaec. Obstet., 41:202,1925.

25. Davidson,E.C.: Sodium chloride metabolism in cutaneous burns and its possible significance for a rational therapy. Arch, Surg., 13:262, 1926.

26. De Roth,A.: Plastic repair of conjunctival defect with foetal membranes. Arch, Ophth. 23:522,1940.

27. Dino,B.R.; Eufemio,G.; Davilla,M. et al: The use of foetal membrane Homografts in the local management of burns. J. Phil. Med.Assoc., 41:890,1965.

28. Dino,B.R.; Eufemio,G.; Devilla,M. et al: Human amnion: The establishment of an amnion bank and its practical applications for Surgery. J. Phil. Med. Assoc., 45:230,1952.

29. Douglas,B.: Homografts of foetal membrane as a coverning for large wounds especially those from burns: An experimental and clinical study. J. Tenn. Med.Assoc.,45:230,1952.

30. Douglas,B.; Convey,H.; Stark,R.B.; et al: The fate of homologous and heterologous chorionic transplants as observed by the transparent tissue chamber technique in mouse. Plast. Recons. Surg., 13:125,1954.

31.a. Dupuytren,G.: Lecons orales dee clinique chirurgicale, Paris Bailliore. Vol 1 p 413 & Vol. 2 p-1, 1832.

31.b. Dupuytren,G.: Clinical lectures in surgery delivered at Hotel Dieu in 1832 (Sidney Doane A transt) Bostan centre, Hender & co., p. 236, 1832.

32. Dzondi,K.H.(1816): quoted by Hauben,D.J.; Yanai,E.; Mahler,D.: On the history of treatment of burns. Burn, 7:383,1981.

33. Eade,G.G.: The relationship between granulation tissue bacteria and skin grafts in burned patients. Plast Recons. Surg., 22:42, 1958.

34. Earle,J. (1799); quoted by Hauben, D.J.; Yanai,E.; Mahler,D.: On the history of the treatment of burns. Burn, 7:383,1981.

35. Ebers,G.(1875): quoted by Hauben, D.J.; Yanai, E.; Mahler, D.: On the history of the treatment of burns. Burn, 7:383, 1981.

36. Elhans,I.P. et al : Role of collagen sheet in the management of burn wound. A thesis for M.S. examination Agra University, Agra, 1978.

37. Fabricius,W.H.(1967):quoted by Hauben,D.J.; Yanai,E.; Mahler, D.: On the history of the treatment of burns. Burn, 7:383,1981.

38. Fox,C.L.Jr.; Rappole,B.W.; Stenford,W.T.: Control of psedomonas infection by silver sulphadiazine, Surg. Gynaec. Obstet., 128:1021, 1969.

39. Fox,C.L. Jr.; Rappole,B.W.; Stenford,W.T.: Silver sulphadiazine for control of burn wound infection. International surgery, 60:5,1975.

40. Galask,R.P.;Snyder,I.S.: Antimicrobial factors in amniotic fluid. Ann. J. Obstet. Gynaec., 106:59,1970.

41. Girdner,J.H.: Skin grafting with graft taken from the dead subjects. Med. Rec. N.Y., 119:1881.

42. Gupta,A.K.; Chaturvedi,S.N.; Samuel,K.C. et al: Preliminary reports of use of collagen sheets as a primary dressing over wound with skin loss. Paper presented at the annual conference (Plastic Surgery Section) held at Trivendrum Feb./March, 1974.

43. Hansen,E.; Troensegaard: Amniotic grafts in chronic skin ulceration. Lancet, 1:859,1950.

44. Hansen,E.; Troensegaard (1956): quoted by Agarwal,V.K.,1982.

45. Hensen,E.; Troensegaard: Peripheral vascular diseases treated by amnion implant: A vreview of 100 cases. Lancet 1:570, 1960.

46. Hebra,F.V.: Hebra on diseases of the skin. London, (Fagga, C.H. transl, 1860, Vol -I. pp. 316).

47. Hebra,F.V.(1861): quoted by Hauben,D.J.; Yanai,E.; Mahler, D.: On the history of the treatment of burns. Burn, 3:383,1981.

48. Heister,L(1743): quoted by Hauben,D.J.; Yanai,E.; Mahler,D.: On the treatment of burns. Burn, 3:383,1981.

49. Idem: Predicting skin graft survival. J. trauma,9:374,1971.

50. Ivanova,S.S.: The transplantation of skin from dead body to granulating surface. Ann, Surg.,12:354,1890.

51. Jain,R.K. et al: An enquiry into role of collagen based surgical in the wounds and burns healing. Thesis for M.S. examination, Meerut University, Meerut, 1976.

52. James,J.H. and Eatson,A.C.: The use of opsite, a vapour premeable dressing on skin graft donor sites. Brit. J. Plast Surg, 28:107,1975.

53. James,A.O.; Neel, Jr. et al: The extended use of skin homografts Arch. Surg., 99:263,1969.

54. Junzekovic,Z.: Present clinical aspects of burns. Mariber, 99,215,1968.

55. Jenner,J.A.: The grafting of preserved foetal membrane to denuded skin surface. Marquette University Milwaukee, Wisconsin.

56. John,D.; Trelford and Marilyn. Trelford-Souder: The amnion in surgery past and present. Ann. J. Obstet. Gynaec., 134(7):833,1978.

57. Jullian, A. Sterling: Use of amniotic membranes to cover surface defects due to flame burns. Ann. J. Surg. 91:940,1956.

58. Katnstan (1941): quoted by Agarwal, V.K.,1982.

59. Kentish,E.: An Essay on burns, London, 1817,pa.102.

60. Kornberg,J.; Bums,H.E.; Kofesjiam,R. et al: Ultra thin silicone polymer membrane: A new synthetic skin substitute. Trans. Ann. Soc. Artif. Inter. Organ, 18:39,1972.

61. Kothari,P.: Total glossectomy and repair with amniotic membrane (Preliminary observations). India Med. Assoc. J., 62:87,1974.

62. Kubani,A.: Trapianto et' amnion sterile ottennto dal teglio cesario. Ann. Ital. Clir, 25:10,1948.

63. Lamke, L.O.; Nilson,G.E. and Reithner,H.L.: The evaporative water loss from burns and the water vapour permeability of grafts and artificial membranes used in the treatment of burns. Burn, 3:159,1977.

64. Liedberg, N.C.F.; Reiss,E. and Artz, C.P.: Infection in burns 111. Septicaemia, A common cause of death. Surg. Gynaec., Obst., 99:151, 1954.

65. Lusgarten (189): quoted by Hauben, D.J.; Yanai,E.; Mahler, D.: On the history of the treatment of burns. Burn, 7:383,1981.

66. Martin,C.; Roben,M.C.: The effect of human amniotic membrane on the bacterial population of infected rat burns. Ann. Surg., 173:144,1973.

67. Martin,C.; Roben,M.C.et al: clinical experience with amniotic membrane as a temporary biological dressing. Year book of Plast. Recons. Surg., 1976.

68. Massee,J.S.; Symmonds,R.E.; Dockorty,M.B. et al: Use of foetal membrane as replacement for pelvic peritoneum after pelvic exenteration in the dog. Gynaec.Obstet, 23:407,1962.

69. Miller,J: Early homografts of second degree burn wounds. Clin Plast. Surg., 1:563, 1974.

70. Monerief,J.A.: Topical antibacterial therapy for burn wounds. Clin. Plast Surg., 1:563,1974.

71. Monerief,J.A. et al: The management of burns I general consideration and sulfamylon method II the silver nitrate, method.

72. Morris,P.J.; Bondoc,C. and Burke,J.E.: The use of frequently changed skin allografts to promote healing in the non healing infected ulcers. Surgery 60:13,1966.

73. Moyer,C.A.; Brontano,L.; Gravenes,D.L. et al: Treatment of large human burns with 5% silver nitrate solution. Arch. Surg., 90:812,1965.

74. Mehta,N.N.; Parekh,J.N.; Bhatnagar,D.; Rai Prahalad Human amniotic membrane as a biological dressing in burn wounds Journal of IMA Vol. 81, 11,12 Nov., 1983.

75. Oppenheimer,L.S.: The treatment of burns, N.K.Med.J., 84:646,1906.

76. Pickerell, K.L.: A sulfonamide film for use of as a Surgical dressings. Bull John Hopkins Hosp., 71:304,1942.

77. Pigeon,J.: Treatment of second degree burns with amniotic membranes. Cem. Med. Assoc. J., 83:844,1960.

78. Pinkerton,M.C.: Amnioplastin for adherent digital flexer tendons. Lancet, 1:70,1942.

79. Pollock:quoted by Fresh water, M.F. and Krizek,T.J.: Skin grafting of burns. A centennial.J. Trauma. 11:862,1971.

80. Pringle,R.: Amniotic implantation in peripheral vascular disease. Lancet, 1:77,1963.

81. Rappaport,I.; Pepino,A.T.; Dietrick,W.: Early use of xenografts as a biologic dressings in burn trauma. Ann. J. Surg., 120:144,1970.

82. Reid,A.: The treatment of burns and other surface wounds. Lancet, 1:677, 1898.

83. Rhazes (1556): quoted by Hauben,D.J.; Yanai,E.; Mahler,D.: On the history of the treatment of burns. Burn,7:383,1981.

84. Robsen,M.C.; Krizek,T.J.; Koss,N. et al: Amniotic membrane as a temporary wound dressing. Surg.Gynaec. Obstet, 136:904,1973.

85. Rowling (1958):quoted by Hansen. Traneugaard, 1960.

86. Sabella,N.: Use of foetal membranes in skin grafting: Med. Rec., N.Y., 83:478,1913.

87. Salisbury,R.E.; Wilmore,D.W.; Silverstein,P. et al: Biological dressing for skin grafting donor sited. Arch Surg., 106:705,1973.

88. Saxena,K.K.: Collagen Sheet and Amniotic membrane application over superficial burn-A comparative study. A thesis for Master of Surgery. Bundelkhand University,Jhansi, 1983.

89. Sharma et al: Evaluation of use of presserved skin homografts in patients of burns. Thesis for M.S. examination surgery. Agra University, Agra, 1978.

90. Shanker,M.L.: A clinical study on the use of collegen sheet in the management of burn wounds. Thesis for M.S. examination. Surgery. Banglore University, Banglore,1975.

91. Shede,M., VII Vereins chronik: Arztlicher verein zu Hamburg. Sitzung von 25, Januer 1881, Dtrch Med Wochenchr, 7:352,1881.

92. Shuck,J.M.; Pruitt,B.A.; Moncreif,J.A.: Homografts skin for wound coverage. A study in versatility. Arch. Surg., 78:472,1969.

93. Silverstain,P.; Curreri,P.W. and Munster,A.M.:Evaluation of fresh viable porcine, cutaneous xenografts as a temporary burn wound cover, Annual Progress report, U.S. Army Institute of surgical Research, 1970-71.

94. Sinha,R.M.; Verma,P.K. and Madam,P.: Collagen sheet as a biological dressings in burns. Ind. J. Plast Surg., 5(2):1972.

95. Sneve,H.: The treatment of burns and skin grafting. J. Am. Med. Assoc., 45:1,1905.

96. Song,I.C.; Bromberg,B.E.; Mohni,M.P. et al: Heterografts as biological dressings for large, skin wounds.Surgery, 59:576,1966.

97. Tavis,M.J.; Thornton,J.W.; Harnvey, J.H. et al: Graft adherence to the de-epithelized surfaces: A comparative study. Ann. Surg., 184:594,1876.

98. Thornton,J.W.; Tavis,M.J.; Harnvey,J.H. et al: Grafts adherence to wound surface: Collagen fibrin interactions. Burns, 3:23,1977.

99. Tommasoli (1897);quoted by Agarwal,V.K., 1982.

100.a.Trelford,J.D.; Anderson,P.G.; Hansen,F.W. et al: Amnion autograft and allograft as a cover for skin defects in sheep, J. Med. 31:81,1972.

100.b.Trelford,J.D.;Anderson,P.G.; Hensen, F.W. et al: Considerations of the amnion as an autografts and allografts in sheep. J. Med., 31:231,1972.

101. Townsend,P.L.G.: The quest for a cheap and painless donor site dressings. Burns, 2:82,1977.

102.a.Trelford,J.D.;Hensen,F.W. and Anderson,D.G.: Amniotic membrane as living surgical dressing in human patients. Onchology, 28:358,1973.

102.b.Trelford,J.D.; Anderson,D.G.; Hansen,F.W.: Amniotic membrane used for radical vulvectomies, Gynaec. Obstet., 12:1,1973.

102.c.Trelford,J.D.; Hansen,F.W.; Anderson,D.G. et al: Implanted amniotic membrane as an autograft and as an allograft, J.Med. 6:169,1975.

103.a.Trelford,J.D.; Hansen,F.W.; Anderson,D.G. et al: Amnion autograft, permanent structure, J.Med., 6:243,1975.

103.b.Trelford,J.D.; Hansen,F.W.; and Anderson,D.G.: Wound healing and the amniotic membrane, J. Med. 6:383,1975.

104. Trelford-Souder,M. and Trelford,J.D.: Replacement of the peritonium with amnion following pelvic exenteration. Surg. Gynaec., Obstet., 145:699,1977.

105. Trelford Souder,M. et al: use of allograft amniotic membrane for control of intra abdominal adhe-sions.J. Med., 9:273,1978.